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Low seroprevalence of systemic cysticercosis among patients with epilepsy in Kerala — South India

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Abstract
Purpose: Neurocysticercosis (NCC) is considered to be rare in Kerala state, India, although it is an important cause of epilepsy in many other parts of India. Our objective was to test this notion by determining the seroprevalence of cysticercosis (CC) in an unselected sample of persons with epilepsy and comparing it to that of persons without epilepsy living in Kerala.
Methods: Individuals with active epilepsy (AE) who had never resided outside Kerala state for more than one month and were attending our center for epilepsy care constituted the cases. Sex-matched persons without epilepsy who had never resided outside Kerala state for more than one month constituted the controls. The demographic details, occupation, and food habits (for the cases and controls), as well as clinical characteristics and imaging (for cases only) were recorded. Sera separated from blood drawn by venipuncture from the cases and controls were assayed for cysticercal antibodies by enzyme-linked immunoelectrotransfer blot (EITB).
Results: Of the 80 persons with AE, 12 were seropositive for cysticercus antibodies (15%; 95% CI: 8.8—24.4); among the 68 controls, 7 were seropositive (10.3%; 95% CI: 5.1—19.8). The odds ratio (OR) for seropositivity in the epilepsy group (1.54) was not statistically significant (95% CI: 0.6—4.2). Among the 69 patients who had a brain computed tomography (CT) scan or magnetic resonance imaging (MRI), none had

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features diagnostic of NCC. Gender, diet (vegetarian vs non-vegetarian, consumption of raw vegetables), drinking water status (clean vs unclean), residence (rural vs urban), exposure to manure, and animal rearing including pigs did not have any association with seropositivity.
Conclusion: Among the residents of Kerala, most epilepsy is not related to cysticercosis.

Introduction

Neurocysticercosis (NCC) is caused by the larval stage of the tapeworm *Taenia solium* in the nervous system. It is the single most common (26.3—53.8%) cause of acquired epileptic seizures in India and other developing countries [1,2]. NCC is now an emerging health problem in industrialized countries due to increased population movement and migration [3]. Although NCC presents as seizures in nearly 70% of patients [4], there are only a few case—control studies on the prevalence of NCC among patients with active epilepsy (AE). The prevalence of NCC in different studies available in India and other countries vary widely according to the methodology. The socioeconomic and cultural-religious backgrounds of the population are likely to influence the prevalence of NCC. It is generally considered that NCC is uncommon in the state of Kerala, where educational and health standards are high, and in Kashmir, where people do not rear pigs or eat pork due to religious customs [5,6].

There are limitations in establishing NCC as the cause of epilepsy in community studies. Brain imaging by computed tomography (CT) scan or magnetic resonance imaging (MRI) is most likely the best method to diagnose NCC but may not be feasible in community studies because of their high cost and limited availability. Seroprevalence for cysticercosis can be estimated by ELISA or enzyme-linked immunoelectrotransfer blot (EITB) techniques. The estimation of antibodies by EITB has higher sensitivity than ELISA (92.2 vs 51.6%) when serum is sampled [7]. Most studies in India and other countries indicate increased seroprevalence among persons with epilepsy [1]. We tested the hypothesis that there is no increase in the seroprevalence of cysticercus antibodies in persons with epilepsy compared to those without epilepsy in Kerala state. We estimated the seroprevalence of cysticercal antibodies by EITB in an unselected sample of persons with AE attending a tertiary hospital in Kerala State and compared it with the value for sex-matched persons without epilepsy.

Materials and methods

This study was carried out in Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Trivandrum, Kerala, a tertiary referral center for epilepsy and other neurological disorders. The samples were analyzed at Christian Medical College (CMC), which is also a tertiary referral center at Vellore, Tamil Nadu. The study was conducted between January 2010 and October 2011.

The selection criteria for the epilepsy group were persons with active epilepsy and 18—60 years old. Epilepsy was defined as an enduring predisposition to generate seizures due to recurrent and unpredictable interruptions of normal brain function [8]. The diagnosis of epilepsy was confirmed by history, while the etiology was determined by clinical examination and data from EEG, CT, or MRI abstracted from the patients' medical records. Active epilepsy was defined as two or more seizures in the past five years or a history of recurrent seizures and currently being seizure free while using antiepileptic drugs [9]. All had at least one year duration of epilepsy prior to enrolment. The comparator group included sex- and age-matched persons without epilepsy. Both groups should have lived continuously in Kerala state and not traveled outside the state for more than one month in their lifetime. Migrant populations were excluded. Controls were identified from the families of epilepsy patients. Controls were also evaluated by history and examination. A standard questionnaire in the local language (Malayalam) regarding food habits (consumption of salads and uncooked vegetables and meat, particularly pork), agricultural activities, and pig/cattle/pet rearing was administered to all patients and controls. The study protocol was approved by the Institutional Ethics Committee and informed consent was obtained from every subject in the local language. The investigators analyzing the serum samples were blinded to the clinical details.

Five milliliters of venous blood was drawn from cases and controls; the serum was separated and stored at −70 °C until assay. Blinding the containers was performed by labeling with dummy numbers after masking the identity by standard coding methods, and the codes were only available from the principal investigator. Sera were transferred frozen to the Neurochemistry Laboratory, Department of Neurological Sciences at Christian Medical College, Vellore, for assay of cysticercus antibodies by EITB, as described in a previous study [10]. Briefly, lentil lectin-specific *T. solium* metacestode glycoproteins (250 ng/mm gel) subjected to non-reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (4—20% gels) were electro-transferred onto polyvinylidene fluoride membranes. The blots were blocked with 5% fat-free casein in phosphate-buffered saline, incubated with 100× diluted sera, and then incubated with goat anti-human immunoglobulin G and rabbit anti-goat immunoglobulin G-horseradish peroxidase and developed with H_2O_2/diaminobenzidine. The blots were analyzed independently by two laboratory personnel, and sera were considered positive for cysticercus antibodies on reaction with one or more *T. solium* glycoproteins of molecular weights 50, 38—42, 24, 21, 18, 14, and 13 kDa [11].

Statistical analysis

Data entry and analysis were completed using the SPSS 17 (Chicago, IL) statistical package. Crude odds ratios were calculated to estimate the relative risk. The distribution of independent variables among case and control groups was analyzed by the Pearson Chi-Square, Fisher Exact Test, and *t*-test. Differences were considered statistically significant at $P < 0.05$.

Results

A total of 80 persons (50 females and 30 males) with AE were enrolled in the study. Their seizures were classified as generalized for 25 (31.2%) and partial for 55 (68.8%). All had at least one year of epilepsy prior to enrolment, and none were treatment naive. CT scan or MRI of the brain was available for 69 out of 80 patients (86.2%), of which 23 (33.3%) were abnormal (mesial temporal sclerosis 8, atrophy 2, tuberculoma 3, arachnoid cyst 4, gliosis 2, cavernoma 1, infarct 2, cortical malformation 1). None of the patients with epilepsy had lesions suggestive of NCC. The control group had 68 persons (41 females and 27 males). There was no significant difference between the cases and controls with regard to rural domicile (73% vs 74%), access to clean drinking water (94% vs 98%), exposure to manure (27% vs 25%), uncooked vegetable consumption (89% vs 94%), and non-vegetarian food consumption (90 vs 93%). The mean age in years of the control group (39.2 ± 14.6) was higher than ($p = 0.01$) that of the patients (33.6 ± 10.2). Animal handling/rearing was significantly higher ($p = 0.001$) among the controls (85%) than among the patients (37%). Although the control group differed from the cases with respect to age and exposure to animals, the differences were not likely to influence the hypothesis.

Serologic results

EITB was positive for 12 of the 80 cases (15%, 95% CI: 8.8—24.4) and 7 of the 68 controls (10.3%; 95% CI: 5.1—19.8). Among the 12 positive patients, 8 had a single band, one had two bands, 3 had three bands, and none had four bands. Among the 7 positive controls, 2 had a single band, 2 had two bands, 2 had three bands, and one had four bands. The odds ratio for seropositivity for the epilepsy cases (1.5, 95% CI .57—4.2) was not statistically significant. Within the epilepsy group, there was no statistically significant difference in the seropositivity for the variables: gender (male vs female), residence (rural vs urban), diet (vegetarian vs non-vegetarian), consumption of raw vegetables, access to drinking water (clean vs unclean source), and animal rearing including pigs (Table 1). There was no association between seropositivity and the type of seizure (generalized vs partial).

Discussion

The key observation in this study is that the prevalence of cysticercal antibodies in an unselected sample of individuals with epilepsy living in Kerala was not significantly different from that of persons without epilepsy living in the same geographic region. There was no difference in the seroprevalence according to gender, habitat, or occupation. Furthermore, none of the 69 individuals with epilepsy for whom imaging data were available had evidence of NCC.

Based on the seroprevalence and negative findings on imaging, we conclude that neurocysticercosis is not an important cause of epilepsy in native Keralites. Prevalence of cysticercosis varies widely depending upon the methods of diagnosis and the geographic location (Table 2). In general, studies utilizing CT or MRI have the highest

Table 1 Demographics,personal data and seroprevalence of cysticercal antibodies in people with epilepsy and controls.

	Controls	Patients	*p* Value
Number	68	80	
Mean age (years)	39	34	.01
Non vegetarians (%)	93	90	.77
Access to clean water (%)	98	94	.22
Rural population (%)	74	73	1.0
Uncooked vegetable consumption (%)	94	89	.77
Exposure to manure (%)	25	27	.85
Exposure to pig rearing (%)	4	0	.09
Seroprevalence of cysticercal antibodies (%)	10.3	15	.47

positivity, followed by detection of cysticercal antibodies in the serum using EITB and ELISA techniques [1,22,26].

Earlier studies utilizing clinical evaluation or imaging had failed to detect NCC as a cause of epilepsy in Kerala [5,12]. This is the first case—control study using serological techniques that confirms that cysticercosis is not a common cause of epilepsy in Kerala state, unlike Tamil Nadu or Andhra Pradesh, locations where it accounts for 38—45% of epilepsy [1,13]. The seroprevalence of cysticercus in this study (10% in the comparator group and 15% in the epilepsy group) is not different from the seroprevalence in the population of Tamil Nadu (13%) [1] or Chandigarh (17.3%) [17], where approximately one-third of epilepsy is attributed to NCC. One possibility for the low levels of the disease, in spite of the comparable seroprevalence, could be that the immune response had prevented its further evolution to NCC. EITB has a low specificity compared to imaging modalities with regard to the ascertainment of NCC. Our results also support this finding, as 15% of our epilepsy cases were EITB positive, but none had imaging evidence of NCC.

The relative rarity of cysticercosis as a cause of epilepsy in Kerala state is of public health importance. The people of Kerala share ethnicity, socio-economic, and religious backgrounds with those living in other south Indian states. A good proportion of the vegetables, food grains, and meat products consumed here are brought in from the neighboring states where there is a higher prevalence of NCC (Table 2). With these shared characteristics, one would expect the prevalence of NCC in Kerala state to be similar to that in the neighboring states. In contrast to these shared characteristics, Kerala has a higher literacy rate (93.2%) and better health-related vital statistics [14,15]. The chain of infection in taeniasis involves the intermediate host, the pig, while that of NCC does not involve pigs and is essentially due to fecal-oral contamination. According to the National Family Health Survey III, (2005—6) 96% of households in Kerala have proper toilet facilities, while in the adjoining states of Tamil Nadu and Karnataka, only 43% and 46% of households have this facility, respectively [16]. This possibly leaves little chance for *Taenia* ova to be ingested through food in Kerala. The higher standards of sanitation especially the

Table 2 Burden of cysticercosis in various Indian states.

Place	Type of study	Sample	Technique	%
Kerala (present study)	Hospital	Epilepsy	EITB	15
			CT/MRI	0
		Controls	EITB	10.3
Vellore 2006 (India) [1]	Population	Epilepsy	EITB	13
			CT	28.4
			NCC*	34
Chandigarh 2006 (India) [17]	Population	Unselected	EITB	17.3
Lucknow 2010 (India) [18]	Population	Unselected	ELISA	3.4
Pondicherry 2005 (India) [19]	Blood donors	Unselected	ELISA	6.48
New Delhi 1994 (India) [20]	Hospital	Epilepsy	MRI	24

CT = computed tomography; EITB = enzyme linked immunoelectrotransfer blot; ELISA = enzyme-linked immunosorbent assay; MRI = magnetic resonance imaging; NCC* = neurocysticercosis as per criteria laid down by Del Brutto et al. [28].

Table 3 Comparison of burden of cysticercosis with data from other countries.

Place	Type of study	Sample	Technique	%
Kerala, India (present study)	Hospital	Epilepsy	EITB/CT or MRI	15/0
		Controls	EITB	10.3
Ecuador 2005 [21]	Population	Epilepsy	EITB/CT/NCC*	33/26.3/33
		Control	EITB/CT	8.3/5.2
Bolivia 2005 [22]	Population	Epilepsy	EITB/CT/NCC*	18.6/29.5/27.4
Peru 2003 [23]	Population	Unselected	EITB	14
Brazil 2002 [24]	Population	Unselected	EITB	1.6
Indonesia 1999 [25]	Population	Unselected	ELISA	1.65
Columbia 1998 [26]	Hospital	Epilepsy	EITB/CT	12/13
Korea1993 [27]	Population	Epilepsy	ELISA	4
		Control	ELISA	2.1

CT = computed tomography; EITB = enzyme linked immunoelectrotransfer blot; ELISA = Enzyme-linked immunosorbent assay; MRI = magnetic resonance imaging; NCC* = Neurocysticercosis as per criteria laid down by Del Brutto et al. [28].

availability of protected toilets reduce the risk of ova contaminating the food or water sources. Salads, uncooked vegetables, and uncooked dishes are not part of Kerala cuisine. Traditionally, the people in Kerala consume all food items, including drinking water, after cooking or boiling, which further reduces the survival of live parasites. Lastly, the burden of stray pigs is very low in Kerala, which in turn limits the taeniasis cycle. It appears that the rarity of epilepsy associated with cysticercosis in Kerala state is multifactorial and dependent on the sociocultural characteristics, food habits, and immune status of the people. Further studies in this field could potentially lead to methods to contain NCC in different parts of the world (Table 3).

Conclusion

Among the residents of Kerala, most epilepsy is not related to cysticercosis, unlike in other states of India.

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Conflicts of interest

The authors declare they have no conflicts of interest.

Ethical approval

The study had the approval of the Institutional Ethics Committee of the Sree Chitra Tirunal Institute for Medical Sciences and Technology.

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